



Occurrence of brucellosis in small ruminants slaughtered in Lafia central abattoir, Nasarawa State, Nigeria

CA Agada^{1*}, AJ Ogugua² & EJ Anzaku³

1. Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria
2. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
3. Department of Veterinary Pathology and Microbiology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria

*Correspondence: Tel.: +2348036506966; E-mail: caysla@gmail.com

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Abstract

Brucellosis caused by *Brucella* species is a disease of economic and public health importance worldwide. Although present in Nigeria, there is a paucity of information regarding the occurrence of the disease in small ruminants in Nasarawa State. A cross-sectional study was therefore carried out to determine the seroprevalence of the disease using Rose Bengal test (RBT) and competitive enzyme-linked immunosorbent assay (cELISA) among small ruminants slaughtered in the Lafia central abattoir. Of the total of 324 small ruminants (sheep and goats) sera collected and tested, 68 (21.0%) and 19 (5.9%) were positive by the RBT and cELISA tests, respectively. The prevalence of 23.8% (61/256) for RBT and 7.4% (19/256) for cELISA as well as 4.4% (3/68) for RBT and 0% (0/68) for cELISA were recorded in goat and sheep sera, respectively. Brucellosis is prevalent in small ruminants with that in goats being more than that of sheep slaughtered at the abattoir in Lafia, Nasarawa State. This is of public health importance to individuals that have regular contact with small ruminants in Nasarawa State. A coordinated surveillance of the disease among the livestock population in the state should be conducted.

Keywords: Brucellosis, Goats, Lafia, Nasarawa State, Sheep, Small ruminants

Introduction

Brucellosis is a disease of animals caused by bacteria of the genus *Brucella*. It is a zoonotic disease which occurs in different species of animals including cattle, sheep, goats, dogs, pigs and many wild species. The disease is ubiquitous although well controlled in most developed countries. It is endemic in developing countries due to lack of well-structured control programmes and inadequate resources (Ayoola, 2014; Kaltungo *et al.*, 2015). The disease is noted for its economic effects in the form of reduced milk production, abortion, infertility, sterility, reduced parity and in humans results in loss

of man hours (Swai & Schoonman, 2010; Adamu *et al.*, 2012; Mai *et al.*, 2012). It therefore impacts negatively on the economy and public health of endemic countries. The primary causative species are *Brucella abortus*, *B. melitensis*, *B. suis*, *B. neotomae*, *B. canis* and *B. ovis* which are responsible for the disease in cattle, goat, pig, desert wood rat, dog and ram, respectively (Whatmore, 2009). While the first four species are known as the smooth *Brucella* species, the latter two are the rough species due to the presence and absence of O-side chains, respectively (Baldi *et al.*, 2000). *Brucella* species have

also been reported in marine animals: *B. pinnipidalis* in the pinnipeds and *B. ceti* in cetaceans (Foster *et al.*, 2007; Dawson *et al.*, 2008). Of the *Brucella* species, *B. melitensis* is the most readily transmissible and pathogenic to humans (Foggin *et al.*, 2000; Marianelli *et al.*, 2007).

The disease is transmitted in animals through sexual transmission, ingestion of infected material, maternal transfer in-vivo or in-vitro and by artificial insemination (Corbel, 2006; Lopes *et al.*, 2010). The presence of the disease in the animal population translates to its occurrence in in-contact humans. It is transmitted to man through contact of abraded skin with infected materials and inhalation. It is, therefore, an occupational disease to livestock workers, laboratory personnel and abattoir workers (Aworh *et al.*, 2013). The disease is also commonly transmitted through the consumption of non pasteurised milk and milk products originating from infected livestock (Sofian *et al.*, 2008). In rare occasions, human-to-human transmission through venereal, childbirth, tissue transplant and blood transfusion have been recorded especially with *B. melitensis* (Vigeant *et al.*, 1995; Falade, 2002; Poulou *et al.*, 2006). In most endemic areas the risk of zoonotic transmission is high due to inadequate measures to protect persons at risk (Bukharie, 2009). Brucellosis is endemic in Nigeria as shown in several serological studies in livestock (Junaidu *et al.*, 2010; Mai *et al.*, 2012; Nanven *et al.*, 2013; Akinseye *et al.*, 2016). The prevalence of 4.9% was recorded among slaughtered cattle in southwestern Nigeria (Ogugua *et al.*, 2015a); 5.5% in donkeys in Borno and Yobe States (Sadiq *et al.*, 2013); 14.1% in cattle screened among herds in Obudu, South-south Nigeria (Nanven *et al.*, 2013); 0.6% in pigs in southeastern Nigeria (Onunkwo *et al.*, 2011) and 9.8% in North-central Nigeria in small ruminants (Bertu *et al.*, 2010).

In Nigeria, rearing of small ruminants is common among the rural populace. However, because these animals are allowed in most cases to roam the streets there is close contact between humans and these animals. This close contact increases the risk of human infection with *Brucella* organisms. Although, there are many studies on brucellosis in Nigeria most of the studies are focused on cattle. Moreover, there is dearth of information regarding the prevalence of the disease in Nasarawa State. To determine the

occurrence of the disease in the State, this study was carried out among small ruminants slaughtered at Lafia abattoir using RBT and cELISA.

Materials and Methods

Study area

The study was conducted at the Lafia central abattoir, Nasarawa State, North-central Nigeria. The State is one of the few that harbour a large population of goats and sheep in Nigeria.

Animal sampling, sample collection and handling

Small ruminants from the Lafia central abattoir were sampled using systematic random sampling. The number of animals slaughtered daily in the abattoir ranged between 100 and 200. Relevant information such as the breed, sex and age of each animal sampled were recorded. Blood samples from the slaughtered animals were collected and allowed to clot and transported to the laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Agriculture, Makurdi where they were centrifuged at 3000rpm for 5 minutes to collect sera. The serum samples were decanted into serum bottles and stored at - 20°C until required for assay. The serum samples were subjected to RBT and cELISA as described by Alton *et al.* (1988) and Perrett *et al.* (2010), respectively.

Rose bengal test (RBT)

RBT was carried out as describe by Alton *et al.* (1988) using antigen sourced from the Animal and Plant Health Agency, Weybridge UK (APHA). Briefly, equal volumes of 30µl of *Brucella abortus* antigen and serum samples were mixed thoroughly with an applicator stick on an enamel plate for four minutes. Appearance of agglutination was recorded as positive while its absence was reported as negative.

Competitive enzyme-linked immunosorbent assay (cELISA)

The cELISA was carried out as described by Perrett *et al.* (2010) using the cELISA test kit sourced from APHA. The reagents in the kit were reconstituted and test carried out according to the instruction of the manufacturers. The optical density (OD) was

measured at 450nm using microplate ELISA reader. A positive/negative cut off was calculated at 60% of the mean of the conjugate control wells. The samples

Table 1: Summary of results of RBT and cELISA tests in goats and sheep

| Test | Positive (%) | Negative (%) | χ^2 | OR | 95%CI | P-value |
|--------|--------------|--------------|----------|-----|-----------|---------|
| RBT | 64 (19.8) | 260 (80.2) | | 3.9 | | |
| | | | 26.13 | | 2.25-6.74 | 0.000 |
| cELISA | 19 (5.9%) | 305 (94.1) | | 1 | | |

that recorded OD less than the 60% cut off were positive, and those above were negative.

Data analyses

Data generated from the study were analysed using STATA version 12 software. The differences in the different groups as well as the two tests used in the study (RBT and cELISA) were tested with chi-square statistics for categorical variables, Fisher Exact Probability test (data obtained from sheep) and unadjusted odds ratio (OR). Significant associations were set at the value of $p < 0.05$.

Results

Out of the 324 small ruminants (goats and sheep) screened for brucellosis, 64 (19.8%) and 19 (5.9%) were found to be seropositive using RBT and cELISA, respectively. The prevalence of 23.8% and 7.4% were recorded among the goat population with RBT and cELISA, respectively with a significant difference occurring between the RBT and cELISA tests ($\chi^2=26.13$; $P= 0.000$; OR: 3.9; 95% CI: 2.25-6.74) (Table 1). The breed specific prevalence in the goat was 22.7% and 6.13% in the West African Dwarf (WAD) and 25.8% and 9.68% in the other breeds. Within the sexes, 32.14% and 7.14% prevalence was recorded in the males and 21.5% and 7.50% among the females. While the prevalence among the older goats (>1year) was 25.0% and 7.21%, that in the younger ones (≤ 1 year) was 18.75% and 8.33% with the RBT and cELISA, respectively (Tables 2 and 3). In the sheep, the prevalence of 4.4% and 0% were recorded with the RBT and cELISA, respectively. Only the WAD (5.36%), females (5.36%) and older animals (4.41%) were found seropositive with the RBT while the cELISA recorded no positive case (Tables 4 and 5).

Discussion

The prevalence of 19.8% as recorded by the RBT in this study shows that brucellosis is enzootic in small ruminants slaughtered in Lafia abattoir. This is of public health importance to individuals who have

Table 2: Prevalence of brucellosis in goats as measured by the RBT

| Variable | Characteristic | Seropositivity | | | | χ^2 | p-value |
|----------|----------------|------------------|------|-------------------|------|----------|---------|
| | | Positive n=61 | % | Negative n=195 | % | | |
| Overall | | | 23.8 | | 76.2 | | |
| Breed | *Others | 24 | 25.8 | 69 | 74.2 | 0.913 | 0.633 |
| | WAD | 37 | 22.7 | 126 | 77.3 | | |
| Sex | Male | 18 | 32.1 | 38 | 67.9 | 2.602 | 0.107 |
| | Female | 43 | 21.5 | 157 | 78.5 | | |
| Age | ≤ 1 year | 9 | 18.7 | 39 | 81.3 | 0.876 | 0.349 |
| | >1year | 52 | 25.0 | 156 | 75.0 | | |

*Others = Sahel red and Kano brown

Table 3: Prevalence of brucellosis in goats as measured by the cELISA

| Variable | Characteristic | Seropositivity | | | | χ^2 | p-value |
|----------|----------------|------------------|-----|-------------------|-------|----------|---------|
| | | Positive n=19 | % | Negative n=237 | % | | |
| Overall | | | 7.4 | | 92.58 | | |
| Breed | *Others | 9 | 9.7 | 84 | 90.3 | 1.253 | 0.534 |
| | WAD | 10 | 6.1 | 153 | 93.9 | | |
| Sex | Male | 4 | 7.1 | 52 | 92.9 | 0.008 | 0.928 |
| | Female | 15 | 7.5 | 185 | 92.5 | | |
| Age | ≤ 1 year | 4 | 8.3 | 44 | 91.7 | 0.07 | 0.792 |
| | >1year | 15 | 7.2 | 193 | 92.8 | | |

*Others = Sahel red and Kano brown

regular contact with livestock like the abattoir workers, flock owners as well as the members of the public; who engage in the consumption of unpasteurised milk and their products in areas where these animals were sourced from. Such risk of human infection is at maximum during lambing and kidding periods due to possible contact with the highly infective birth materials from infected animals (EC, 2001). Considering the fact that malaria and typhoid which show clinical signs similar to brucellosis are endemic in Nigeria (Igbeneghu *et al.*, 2009; Eze *et al.*, 2011), many brucellosis cases could be mistaken for these diseases resulting in wrong diagnosis and treatment failures of patients suffering from the disease (Baba *et al.*, 1998; Bahador *et al.*, 2012). The prevalence of 19.8% recorded in small ruminants in this study is lower than 26.5% recorded in sheep flocks in Kaduna (Kaltungo *et al.*, 2015) but comparable to that of an abattoir study (22.93%) in goats in Sokoto (Junaidu *et al.*, 2010). It is however higher than that of other studies: 2.83% in goats screened in selected states in Nigeria (Ogugua *et al.*, 2015b), 9.8% in small ruminants in Plateau State (Bertu *et al.*, 2010), 13.3% in small ruminants slaughtered in abattoirs in Ghana (Jarikre *et al.*, 2014), 9.38% in small ruminants in Ethiopia (Negash *et al.*, 2012); 3.13% in small ruminants flocks in Eastern Ethiopia (Teshale *et al.*,

Table 4: Prevalence of brucellosis in sheep as measured by the RBT

| Variable | Characteristic | Seropositive animals based on RBT | | | | Fisher Exact Probability Test p-value |
|----------|----------------|-----------------------------------|-----|------------|-------|--|
| | | Positive n | % | Negative n | % | |
| Overall | | 3 | 4.4 | 65 | 95.6 | |
| Breed | **Others | 0 | 0.0 | 12 | 100.0 | 0.55 |
| | WAD | 3 | 5.4 | 53 | 94.6 | |
| Sex | Male | 0 | 0.0 | 12 | 100.0 | 0.55 |
| | Female | 3 | 5.4 | 53 | 94.6 | |
| Age | ≤1year | 0 | 0.0 | 5 | 100.0 | 0.79 |
| | >1year | 3 | 4.8 | 60 | 95.2 | |

**Others = Balami, Uda and Yankasa

Table 5: Prevalence of brucellosis in sheep as measured by the cELISA

| Variable | Characteristic | Seropositive animals based on cELISA | | | | Fisher Exact Probability Test p-value |
|----------|----------------|--------------------------------------|-----|------------|-------|--|
| | | Positive n | % | Negative n | % | |
| Overall | | 0 | 0.0 | 68 | 100.0 | |
| Breed | **Others | 0 | 0.0 | 12 | 100.0 | 0.99 |
| | WAD | 0 | 0.0 | 56 | 100.0 | |
| Sex | Male | 0 | 0.0 | 12 | 100.0 | 0.99 |
| | Female | 0 | 0.0 | 56 | 100.0 | |
| Age | ≤1years | 0 | 0.0 | 5 | 100.0 | 1 |
| | >2years | 0 | 0.0 | 63 | 100.0 | |

**Others = Balami, Uda and Yankasa

2006) and 14.7% in small ruminants in Iran (Zowghi & Ebadi, 1985). The prevalence of brucellosis in the area of study may be attributed to the fact that there is no control scheme for, and low knowledge of brucellosis in Nigeria (Onoja *et al.*, 2008; Adesokan *et al.*, 2013). Also, for the fact that brucellosis induced abortion rarely reoccurs in subsequent pregnancies (OIE, 2009), many obviously infected females are retained in flocks in Nigeria (Mai *et al.*, 2012). This results in continued propagation of the disease especially in rural communities where small ruminants are left to roam freely and mate indiscriminately (Bertu *et al.*, 2010; Kaltungo *et al.*, 2015; Ogugua *et al.*, 2015b). In addition, in pastoral communities small ruminants are allowed to graze in common with cattle herds increasing their risk of getting infected with other *Brucella* species (Ocholi *et al.*, 2005). Moreover, movement of livestock within Nigeria and between the neighbouring countries is not controlled resulting in continued transmission of diseases like brucellosis (Ogundipe, 2001).

This study found the prevalence of brucellosis to be higher in goats (23.8%) than in sheep (4.4%) with the RBT. This is similar to what was recorded in other studies (Teshale *et al.*, 2006; Bertu *et al.*, 2010; Negash *et al.*, 2012; Adugna *et al.*, 2013; Jarikre *et al.*, 2014). This may be because goats are more susceptible to *Brucella* infection than sheep and also goats shed the organism in milk and semen for

longer periods (Teshale *et al.*, 2006; CFSPH, 2009b; Adugna *et al.*, 2013). Also, *B. melitensis*, the major cause of brucellosis in small ruminants, is known to readily infect most breeds of goats but the susceptibility to infection with the organism varies to a great extent among different sheep breeds (CFSPH, 2009b).

Also, there was a significant difference between the RBT and cELISA results ($\chi^2=26.13$; $P=0.000$ OR: 3.9; 95% CI: 2.25-6.74) with the RBT recording higher prevalence of brucellosis than the cELISA. The cELISA has been shown to be of lower sensitivity and does not outperform the standard RBT in the diagnosis of sheep brucellosis (Marín *et al.*, 1999). Indeed, the RBT has been advocated as the test of choice in small ruminants in areas such as Nigeria where vaccination is not generally practised (Marín *et al.*, 1999; Ducrotoy *et al.*, 2014). This is because, in the absence of vaccination, RBT is superior to cELISA (Ducrotoy *et al.*, 2014) and the standard set by this test in the diagnosis of brucellosis is yet to be matched by any other serological test (McGivern, 2013). However, the possibility that the RBT positive but cELISA negative samples could be as a result of presence of antibodies to other Gram negative organisms like *Yersinia enterocolitica* 0:9, *Vibrio cholera* and *Salmonella urbana* group N may not be ruled out (Nielsen *et al.*, 1996; Neta *et al.*, 2008; OIE, 2009). There is therefore the need to involve

bacterial isolation in subsequent studies of small ruminant brucellosis in the area.

Though there was no significant difference, the study recorded higher prevalence in the male than female goats with the RBT. This is contrary to other findings (Teshale *et al.*, 2006; Negash *et al.*, 2012; Adugna *et al.*, 2013; Jarikre *et al.*, 2014; Kaltungo *et al.*, 2015; Ogugua *et al.*, 2015b) that recorded higher prevalence in does than in bucks. The higher prevalence in males may be attributed to the fact that although brucellosis transmission from the buck to the doe through natural means is not common (CFSPH, 2009b) due to the vaginal environment which is not conducive to the survival of *Brucella* deposited by the male (Chakrabarti, 2012), but bucks get infected while serving infected does (CFSPH, 2009b). Also, bucks are readily infected when they come in contact with infected semen deposited by other infected males during co-servings of does on heat (Godfroid *et al.*, 2004). In addition, small ruminant males are known to exhibit homosexual behaviours and *Brucella* infection is readily established when the organism is deposited on abraded mucous membranes (EC, 2001; CFSPH, 2009a) an occurrence which is common with anal sex (Ungerfeild *et al.*, 2014).

However, the study was undertaken in the abattoir and therefore not representative of the situation in the local herds/flocks in the area studied. In addition, the animals screened could not be traced to the farms of origin where information about the possible vaccination of these animals could be enquired since small ruminants slaughtered in the area of study had no identification tags. Nonetheless, many studies in Nigeria validly assume non vaccination of animal populations surveyed because in most cases there is no history of certified vaccination against brucellosis and more so, vaccination against the disease is not routinely carried out in local herds (Mukhtar & Kokab, 2008; Onoja *et al.*, 2008; Bertu *et al.*, 2010; Cadmus *et al.*, 2013; Kaltungo *et al.*, 2015). Also, there was no bacteriological confirmation of the disease among the seropositive small ruminants screened since the *Brucella* species responsible for the disease were not isolated. However, serology alone had been used for the study of brucellosis by other workers (Teshale *et al.* 2006; Mukhtar & Kokab, 2008; Onunkwo *et al.*, 2011; Cadmus *et al.*, 2013; Adamu *et al.*, 2014; Jarikre *et al.*, 2014).

Finally, this study found brucellosis to be prevalent in small ruminants slaughtered in Lafia abattoir though not significantly associated with the breed,

sex or age of the animals screened. This may constitute a major risk of infection to individuals in the area as a result of close human-animal interaction in rural communities in Nigeria and other enzootic areas in Africa. Also, this study showed a significant difference between the two diagnostic methods used; therefore we advocate that it is not necessary to include cELISA in serological studies for brucellosis in the area of study except in cases where there are proofs of vaccination against the disease. However, further studies should involve isolation to confirm this as well as identify the *Brucella* species responsible for the disease in small ruminants in the area. In addition, given that slaughter animals were used in this study, subsequent studies in the state should focus on the flocks in their local settings and the risk factors facilitating the transmission of the disease among the animals and between animals and humans.

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